2671-Pos Board B641

Direct Measurement of the Relative Contributions of Turgor Pressure, the Peptidoglycan Cell Wall and Cytoskeletal Filaments to Gram-negative Prokaryotic Cell Mechanics using AFM

Mingzhai Sun[1], Yi Deng[2], Hugo Arellano Santoyo[2], Siyuan Wang[3], Joshua W. Shaevitz[1,2].
[1]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA, [2]Department of Physics, Princeton University, Princeton, NJ, USA, [3]Department of Molecular Biology, Princeton University, Princeton, NJ, USA.

The envelope of a gram-negative bacterium is composed of three layers: the inner and outer membranes and the periplasmic peptidoglycan (PG) cell wall. The PG layer is thought to be the stress bearing structure that determines and maintains cell shape, preventing cells from bursting due to a large inner turgor pressure. In vitro, PG mechanics have been measured with different techniques, yielding a high elastic modulus. However, in vivo such mechanical measurements are complicated by the turgor pressure. On the other hand, direct measurement of a cell's turgor pressure is difficult due to the presence of the PG layer. We are able to overcome these difficulties and simultaneously measure cell turgor pressure and PG mechanics in vivo with an atomic force microscope (AFM). Using a vancomycin sensitive strain of Escherichia coli, we locally induced small membrane blebs at sites of reduced PG integrity. AFM indentation of these membrane-bound blebs directly probes the turgor pressure. By comparing bleb indentation with indentation of the cell body, we can deduce the relative contributions of the PG layer and turgor pressure to the overall cell mechanics. Furthermore, drugs that inhibit bacterial cytoskeletal filaments can be used to evaluate the role of these proteins in modulating cell elasticity.

2672-Pos Board B642

Mechanism of MSP-based Cell Body Retraction in the Amoeboid Sperm of Nematodes

Katsuya Shimabukuro[1], Murray Stewart[2], Thomas M. Roberts[1].
[1]Florida State University, Tallahassee, FL, USA, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

Cell body retraction in the amoeboid sperm *Ascaris suum* is generated by rearrangement and disassembly of the MSP cytoskeleton, without involvement of the motor proteins that pull the cell body forward in actin-based crawling cells (Miao et al. 2003. Science 302:1405). Reconstitution of retraction *in vitro*, which involves shortening of columnar meshworks of MSP filaments called fibers that assemble in cell-free extracts of sperm, has enabled us to explore the biochemical and biophysical basis of retraction. We found that fiber retraction is induced by treatment with low pH buffers with an optimum effect on both the rate and extent of retraction at pH 6.0. 3D correlative light and electron microscopy showed that there is a progressive loss of filament mass during retraction. However, the filament length distribution within fibers did not change significantly suggesting that retraction involves selective loss of filaments rather than uniform disassembly of all filaments in the fiber. Stereology revealed that during retraction filaments tended to rearrange from a random orientation to alignment along the long axis of fiber. (degree of orientation = 20 to 30%). Following the movement of small vesicles trapped filament mesh of the fiber, which appear as ''specks'' under optical microscopy, showed that shortening was faster and more extensive in the newest portion of the fiber. Moreover, we found that specks that started several microns apart could overtake one another but then moved in tandem as the fiber continued to shorten. These results suggest that the pattern of filament rearrangement is not uniform throughout the fiber. We are currently seeking to apply fluorescence speckle microscopy to explore the dynamics of filament rearrangement at higher resolution. Supported by NIH Grant R37 GM29994.

2673-Pos Board B643

FRAP Analysis Combined With A Single-cell Electroporation Technique In Sea-urchin Spermatozoa

Daisuke Takao[1], Shinji Kamimura[2].
[1]The University of Tokyo, Tokyo, Japan, [2]Chuo University, Tokyo, Japan.

In sea-urchin spermatozoa, energy required for flagellar motility is provided by ATP diffusion from mitochondria located at the proximal ends of flagella along with the creatine shuttle system. However, no direct analysis of the diffusion rates inside flagella has been carried out thus far. Using a FRAP (fluorescence recovery after photobleaching) technique, we determined the diffusion coefficients of fluorescein-derivatives (AM esters of calcein, carboxyfluorescein, and Oregon Green; MW 376-623) to be 63-64 $\mu m^2/s$. Although these values are about one third of the estimates that were previously used for theoretical calculations (Tombes et al., 1987), we concluded that the rate of ATP diffusion inside spermatozoa was high enough to support the continuous motility of sea-urchin sperm flagella if creatine shuttle system is working. In order to investigate diffusion properties of fluorescent dyes of a wide range of molecular weights, we used a single-cell electroporation (SCE) technique, which has been developed for nerve cells (Bestman et al., 2006). Using the technique, we succeeded to inject fluorescein dextran of MW 3,000 (3k-FD) into sea-urchin sperm cells. By FRAP analysis, we determined the diffusion coefficients of 3k-FD to be ~30 $\mu m^2/s$, about one half of that of carboxyfluorescein (MW 376), almost consistent with the value estimated from the molecular weights. We also investigated the diffusion properties through the ''neck'' regions, between the head and tail of spermatozoa. When the head region of calcein-loaded spermatozoon was photobleached, slow recovery of head fluorescence along with the decrease of fluorescence signal in the tail region was observed. It suggests that small molecules like calcein (MW 623) can move almost freely through the boundary between the head and tail.

2674-Pos Board B644

System Analysis of the Ciliary Response to Red Light in *Chlamydomonas reinhardtii*

Suphatra Adulrattananuwat, Keith Josef, Jureepan Saranak, Kenneth Foster.
Physics Department, Syracuse University, Syracuse, NY, USA.

To understand signal processing networks within cells we study the motile green alga *Chlamydomonas reinhardtii*. This organism swims with two cilia and has an eye with rhodopsin (peak sensitivity 500 nm, green) controlling the direction of its swimming (phototaxis). Here we report on a red light receptor (peak sensitivity 670 nm) that influences the frequency of ciliary beating, which we monitor by observing a single cell held on a micropipette with a quadrant photodiode. For square-wave on-off stimuli, the recorded beating frequency shows the on-step latency is strongly light intensity dependent, ranging from 700 ms at 1.3 W/m^2 to 200 ms at 50 W/m^2. This long delay suggests a diffusive step in the signaling pathway and contrasts with the sub-milliseconds latency of green light stimuli. The response amplitude is sustained for the full length of the on-step and is also light intensity dependent. However, the off-step latency seems light independent ranging from 400 to 1600 ms. Following the light off, the beating frequency drops more rapidly and farther the greater the prior light intensity. Products appear to be synthesized in proportion to the light intensity, but once the light is off these products in the cilium run out after about a second. Since the beating frequency is feedback regulated, the red-light elevation of the beating frequency is compensated by a counter molecular change in the cilia. Consequently, when these products run out, there is a rapid declined in beating frequency that takes about 10 s to recover. In addition to steps, sine and white-noise stimuli have been used to refine the response function.

2675-Pos Board B645

Abnormal Movement And The Trend Of Flagellar Force Production During Regeneration In *Chlamydomonas Reinhardtti*

Catherine G. Clodfelter, John Yukich, Karen Bernd.
Davidson College, Charlotte, NC, USA.

Several respiratory, digestive, and reproductive disorders result from dysfunctional cilia and flagella. Because the form, strength or frequency of flagella motion is abnormal in these disorders, biological functions performed by the cells utilizing flagella and cilia are debilitated. The causes of debilitation are not known as the internal mechanism for creating the flagella's breast stroke-like motion is still not fully understood. This study uses *Chlamydomonas reinhardtii* flagella as a model system and reports standardization of PSD calibration, laser trapping and cell movement recording methods. When motion in a laser trap is viewed as a trace, natural cell rotation while swimming (caused by flagellar dominance) results in a spirograph-like 'donut' shape. Circle fit analysis programs were written to measure trends in force generation during flagellar regeneration. The percentage of 'donuts' per flagella length increases linearly, similar to the increase in length over regeneration time. However the flagellar force trend shows a significant, unexpected dip during flagella lengthening. Neither the flagella infrastructure necessary to establish dominance nor the causes of the decrease in force are known, thus these data open many new research directions.

2676-Pos Board B646

How Does Stall Force Affect Contractions of a Biological Spring, *Vorticella convallaria*?

Sangjin Ryu[1,2], Paul T. Matsudaira[1,2].
[1]Whitehead Institute for Biomedical Research, Cambridge, MA, USA, [2]Massachusetts Institute of Technology, Cambridge, MA, USA.

The stalk of *Vorticella convallaria*, a sessile peritrich, is considered as a model biological spring for bio-inspired actuators because of its remarkable speed and force. When stimulated, the stalk of *Vorticella* contracts over a few hundreds micrometers in a few milliseconds, and its energy source is not ATP but